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Altered sex ratio in giant fresh water prawn, *Macrobrachium rosenbergii* (de Man) using hormone bioencapsulated live *Artemia* feed

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Abstract

Artemia nauplii cultured for 24 h in lipid-enriched media contained 0, 0.38, 1.67, 0.79, 5.71 and 5.14 ng 17α-methyl testosterone (MT) mg^{-1} dry weight for the control, 5, 15, 30, 50 and 100 mg of 17α-MT L^{-1} of media respectively. Giant fresh water prawn *Macrobrachium rosenbergii* larvae were fed hormone-enriched *Artemia* nauplii for 50 days. A significant difference in sex ratios (0.92, 0.69 and 0.93) was obtained in relation to the control group. These results suggest that further research is needed with regard to standardization of doses and duration of treatments for complete hormonal control of sex differentiation in *M. rosenbergii*.

Keywords: *Macrobrachium rosenbergii*, 17α-methyl testosterone, bioencapsulation, radioimmunoassay, sex reversal

Introduction

While monosex culture technology has become a common practice in fish (Tayamen & Shelton 1978; Macintosh, Verghese & Rao 1985; Lee & Donaldson 2001), very few attempts have been made on crustaceans (Curtis & Jones 1995; Sagi, Milstein, Eran, Joseph, Khalaida, Abdu, Harpaz & Karplus 1997). Sagi, Ra'anan, Cohen and Wax (1986) separated prawn by sex and showed that it was more advantageous to grow all males than a mixed culture in the case of fresh water prawn. The growth of females is hampered by the presence of males indicated by a lower yield (Sagi *et al.* 1986) and the differences in inter- and intra-sex growth rates affect prawn culture causing more variation on body size at harvest, total yield and product value (Malecha 1983a; Hulata, Karplus, Wohlfarth & Halevy 1988). Hence, sex manipulation could be of great use in prawn aquaculture. Unfortunately, sex determination is not clearly understood in most groups of crustaceans (Malecha 1983b; Das & Lakra 1998). Sex in penaeid prawn is determined by a single or small number of loci (Reed & West 1998) and is probably of XO, XY type (Niiyama 1941, 1959, 1983) along with reports on protogynous sex change (Brook, Rawlings & Davies 1994; Abe & Fukuhara 1996). The chromosomal sex-determining mechanism is imperfect because of environment, epigenetic and genetic factors (Malecha, Nevin, Ha, Barck, Lamadrid-Rose, Masuno & Hedgecock 1992).

Sex reversal has been achieved in many fish species using hormone incorporated pelleted diets. However, the hormone treated commercial diets are unsuitable for fish and prawn species that require live diets during the time of sexual differentiation. Hence, many workers in the past have successfully used hormone-enriched *Artemia* as food for sex reversal in fish species (Martin-Robichaud, Peterson, Benfey & Crim 1994; Stewart, Spicer, Inskeep & Dailey 2001; Garret 1989).

The present study was aimed to assess the sex ratios and growth enhancement following the use of hormone-enriched *Artemia* as food, which could lead to a technology of monosex (all males) and enhanced production for fresh water prawn, *Macrobrachium rosenbergii*.

Materials and methods

Animals

The larvae of giant fresh water prawn *M. rosenbergii* were obtained from the indoor hatchery of Central Institute of Fisheries Education, Mumbai and reared in plastic tubs. All the larvae were taken from a single hatching cycle and reared for 5 months during September–January 2002.

Enrichment of *Artemia*

The *Artemia* cysts (Dynasty™, Salt Lake City, UT, USA) were hatched daily for hormone [17α-methyl testosterone (MT), Sigma Chemical Company, St Louis, MO, USA] enrichment using the method described by Sorgeloos, Levens, Leger, Tackaert and Versichele (1986). They were first decapsulated with sodium hypochlorite solution (at 15 mL L^{-1}) for 10 min, filtered through nitex screen and washed with fresh water to remove the traces of chlorine. To ensure complete removal of chlorine, the cysts were treated with 0.1% sodium thiosulphate. These were then transferred to a conical flask containing 4 L of seawater (25 g L^{-1}) with vigorous aeration maintained at 28 °C and kept for 24 h under natural light for hatching. After 24 h, aeration was stopped and the hatched larvae that settled down were removed using nitex screen. The hatching was confirmed microscopically. The *Artemia* nauplii were divided equally in six parts and transferred to glass jars of 2-L capacity for hormonal treatment at five different doses of 17α-MT (5, 15, 30, 50 and 100 mg L^{-1}) for about 24 h. Since the hormone is not soluble in water, it was dissolved in 2 mL of 90% ethanol, the same quantity of ethanol was added to the control group. The emulsified diet was prepared using cod liver oil and egg yolk at 0.8 g L^{-1} and added to glass jars containing *Artemia* nauplii. After 24 h of hormone treatment, the hormone-enriched *Artemia* nauplii were filtered through nitex screen, washed and fed to the prawn larvae in plastic tubs, twice a day starting from 5 days post hatch (PDH) to 54 days post hatch (PDH). The tubs were siphoned daily to remove wastes and 25% of the water was exchanged every 2 days.

Two samples of hormone-enriched *Artemia* nauplii were taken from each treatment at 0, 8, 12, 16, 20 and 24 h for detection of hormone incorporation in the tissue of *Artemia* nauplii. The aeration was stopped to allow settling of nauplii. About 10 mL of sample was taken with pipette, filtered, rinsed with distilled water and kept in preweighed test tubes at − 80 °C until radioimmunoassay (RIA) analysis was done.

After the treatment period (50 days), when the larvae metamorphosed into postlarva, the prawns were transferred to larger tanks (50-L capacity) provided with shelters and fed with hormone-free diet having 40% protein at 5% body weight four times a day. After 120 days, they were sexed by observing the appendix masculina (Sandifer & Smith 1985), and presence of gonopores (Nagamine, Knight, Maggenti & Paxman 1980). To determine the concentration of 17α-MT in prawn tissue after 50 days of feeding, the prawn juveniles were sampled from treatments and control. The prawn juveniles were rinsed in distilled water and placed in preweighed tubes, then frozen, lyophilized and coarsely ground. 17α-MT concentration was determined using RIA.

Experimental design

The experiment consisted of five treatments of 17α-MT: (a) 5 mg L^{-1}; (b) 15 mg L^{-1}; (c) 30 mg L^{-1}; (d) 50 mg L^{-1} and (e) 100 mg L^{-1} for enrichment of *Artemia* and a control. Four replicates were kept for each treatment, each comprising 200 larvae in a plastic tub of 15-L capacity. The temperature and pH of the water varied between 22–28 and 8.3–8.5 °C, respectively, and the tanks were provided with 24 h aeration along with shelters.

Radioimmunoassay

Since steroids are not species specific, RIA for 17α-MT was done following Mukku, Prahalada and Mudgal (1976). We analysed samples in duplicate. Pre weighed samples (prawn muscle, *Artemia*) were taken in a tissue homogenizer, macerated and extracted with 10 mL diethyl ether slowly. The supernatant fluid was collected in a culture tube and the process was repeated twice and then dried at 30–32 °C. The dried extract was re-extracted by dissolving in 1.0 mL of gelatin phosphate-buffered saline (GPBS) and vortexed for 1 min and 100 μL of sample was drawn for hormonal assay. To sample tubes, H^3 (tritium) labeled antigen (methyl testosterone 3-CMO-BSA, New Life Science Products, England, UK) – 0.1 mL (10 000 cpm), antibody (UCB Biproducts SA, Braine-L'Alleud, Belgium) – 0.1 mL (1:70 000) and GPBS – 0.1 mL was added and incubated for 4 h at 4 °C. Then 300 μL of

chilled dextran coated charcoal was added, vortexed and incubated for 10 min at 4 °C, centrifuged at 4000 *g* for 20 min at 4 °C. Scintillation fluid (2.0 mL) was added to supernatant in counting vials and radioactivity was counted in Beckman Counter (Model LS-1701, Beckman Instruments, Fullerton, CA, USA) for 1 min.

Results

The RIA analysis revealed that brine shrimp accumulated 0.38 ± 0.019, 1.67 ± 1.527, 0.79 ± 0.205, 5.71 ± 4.285 and 5.14 ± 4.851 ng of 17α-MT mg^{-1} dry weight of tissue at hormone concentration of 5, 15, 30, 50 and 100 mg L^{-1}, respectively, after 24 h (Fig. 1). The hormone concentration in prawn tissue was found 3.2 ± 0.182, 5.0 ± 0.258, 2.8 ± 0.182, 10.8 ± 0.658 and 20.9 ± 4.744-pg mg^{-1} dry weights at hormone dose of 5, 15, 30, 50 and 100 mg L^{-1} of 17α-MT respectively.

A slightly higher growth was obtained in treatment five (100 mg L^{-1}) but it was not significantly different ($P > 0.05$) from the control. There was no significant effect of hormone doses on growth in juveniles. The maximum survival was observed in control in comparison with hormone treatments. A skewed sex ratio was observed with 30, 50 and 100 mg L^{-1} hormone doses (Table 1), which were significantly different ($P < 0.05$). In all other treatments, these ratios were not significantly affected.

Discussion

The vertebrate androgenic steroids can increase testis size in prawn (Nagabhushanam & Kulkarni 1981), convert ovaries into testis in ocypod crab (Sarojini 1963), delay molting of neonatal organisms and can delay molt frequency in *Daphnia magna* (Mu & LeBlanc 2002). Consequently, a number of experiments have demonstrated the effect of methyl testosterone as a growth promoter along with an effective agent for sex reversal in fish (Macintosh *et al.* 1985). More recently, an improvement in the growth along with sex reversal has been reported in several fish species using 17α-MT (Pandian & Sheela 1995). However, no growth promoting effect of methyl testosterone on the prawns was observed in the present experiment thus corroborating the findings of Antiporda (1986). This is clearly shown in the analysis of variance of mean body length of prawn after 120 days. The sex ratios with 30, 50 and 100 mg L^{-1} MT treatments were significantly different from the control ($P < 0.05$). A

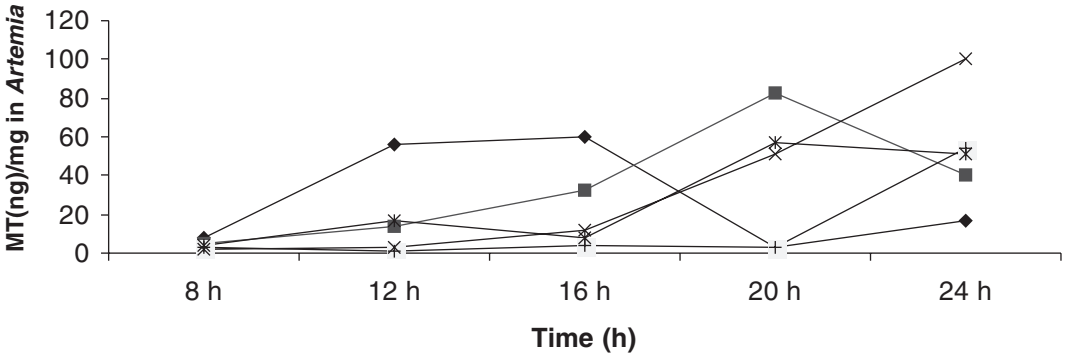

Figure 1 The levels of 17α-methyltestosterone (ng mg^{-1}) in *Artemia* sample. Data represent the mean of duplicate RIA aliquots from each *Artemia* sample.

Table 1 Sex ratios determined phenotypically

Treatment	Duration (days)	Dose of MT mg L^{-1}	No. of prawn sexed	Male:female	Mean % survival
Control	–	0	536	0.41	67 ± 0.456
T1	50	5	330	0.49	41.3 ± 0.504
T2	50	15	453	0.48	56.7 ± 0.439
T3	50	30	396	0.92	49.6 ± 1.369
T4	50	50	340	0.69	42.5 ± 1.925
T5	50	100	328	0.93	41.1 ± 0.735

significant difference in the sex ratios among different treatments was also obtained ($P < 0.05$). Similar observations have been reported by Antiporda (1986) using 25 mg MT kg^{-1} feed in prawn juveniles.

The 17α-MT administered to prawn juveniles via *Artemia* was around 2–20 pg mg^{-1} dry weight. The concentrations of 17α-MT incorporated into *Artemia* tissue were less than that observed by Martin-Robichaud and colleagues (1994). It may be due to the fact that the hormone enrichment started before first feeding at the instar II stage of *Artemia* due to the mouth size of prawn larvae. The concentrations of hormone get reduced by storage and feeding period (Martin-Robichaud *et al*. 1994).

The reduced survival in the juveniles could be due to the role of hormone as antiecdysteroids because the exogenous steroids act as moult inhibiting agents in crustaceans (Olmstead & LeBlanc 2000). The present study did not achieve the expected sex reversal by non-native methyl testosterone but the findings may form the basis for further research in sex determination, gonadal differentiation and monosex production in *M. rosenbergii*.

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